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DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

ON THE LUMINESCENCE OF LUMINOLS VIII: A VERY EFFECTIVE
INHIBITION OF THE LUMINOL REACTION

Following is a translation of an article by
K. Weber and R. Kostelac, Research Depart-
ment, "Fotokemika" Film and Photographic Paper
Factory at Zagreb, Croatia, Yugoslavia, in the
German-language, published in Croatica Chemica
Acta 28(1956), pages 33-39.⁷

The chemoluminescence of Luminol caused
by the oxydizing influence of potassium-ferro-
(III)cyanide may be controlled in such a man-
ner that either a non-catalytic or a catalytic
reaction will result. Both reaction types are
susceptible to inhibiting influences by foreign
additions; hydrochinon causes a very effective
inhibition of the non-catalytic reaction and
cancellation of luminescence in very low con-
centration. Half-value concentration for can-
cellation is 1.10×10^{-7} mol/l and it should be
pointed out that inhibition in this order of
magnitude was thus far observed only in chain
reactions. Other inhibitors show a much weaker
effect. The assumed reaction mechanism of lumi-
nescence will be discussed in the following.

Two basically different types of the Luminol reaction
are known. In one type hydrogen peroxyde serves a dual pur-
pose as oxydation medium and reaction component. Its oxygen
is activated by means of a suitable catalyst (in most cases
a heavy metal complex) and is brought to act on the Luminol
molecule in a manner similar to the peroxydizing process. In
the other type of reaction process an oxydizing medium (po-
tassium-ferro(III)cyanide or hypochlorite) will act directly
on the Luminol, without requiring the catalyst ^[1] numbers
in brackets refer to similarly numbered items in Bibliography

at end⁷. Both types of reaction will display a more or less intensive emission of blue light. In regard to the inhibiting (retarding) of the reaction and cancellation of luminescence we were able to note that the two modifications of this reaction show essentially different characteristics. In the presence of hydrogen peroxyde and a respective catalyst the order of magnitude of the inhibiting effects obtained was comparable to those observed regularly in thermal and photochemical reactions and in the cancellation to fluorescence to dissolved substances [2]. A very effective inhibition, which was noted in the case of non-catalytic reactions, which will be discussed in the following.

Test Methods

The tests were carried out with the photoelectric apparatus described in previous articles. Three series of tests were carried out for each of the reaction compounds containing the following components:

1. Luminol 2×10^{-3} mol/l
NaOH 2.5×10^{-2} mol/l
Potassium-ferro(III)cyanide 4×10^{-4} mol/l
2. Luminol 4×10^{-4} mol/l
NaOH 5×10^{-3} mol/l
H₂O₂ 1.76×10^{-2} mol/l
Potassium-ferro(III)cyanide 4×10^{-4} mol/l
3. Luminol 4×10^{-4} mol/l
NaOH 4.5×10^{-2} mol/l
H₂O₂ 1.76×10^{-2} mol/l
Potassium-ferro(III)cyanide 4×10^{-4} mol/l

The reaction volume in all cases was 50 ml. Test carried out with the components listed under 1. obviously refer to the non-catalytic type of reaction while the tests resulting from 2. and 3. yielded catalytic reactions at different alkaline concentrations. The inhibitors (foreign additions) used were: phenol, hydrochinon, methyl-paramido-phenol-sulfate (metol), anilines and potassium iodide (KI). Measurements were made in relative units (galvanometer deflection) for the intensity of luminescence dependent on the reaction time, with foreign additions present or absent. The light sums of luminescence emissions and the half-value concentrations for inhibition were computed from the test results. (For definition of these terms see publication VII on this subject).

Test Results

It is evident that the above listed reaction compounds are composed in such a manner that the potassium-ferro(III)-cyanide was kept at a constant concentration. With hydrogen peroxyde present, the concentration of the ferruginous complex is equal to the Luminol concentration while the H_2O_2 concentration was chosen at a ratio of nearly 1:50 larger than the other concentrations. This surplus is necessary in order to move the peroxyde into the position of the second reaction component and to permit the ferro-complex to act as a catalyst. Oxidizing of the Luminol will then proceed in accordance with the oxygen resources offered by the peroxyde and will terminate when all the Luminol has been used. In the absence of hydrogen peroxyde a much higher Luminol concentration had to be chosen in order to obtain measurable and comparable test results for luminescence. In this case Luminol is present in five times higher quantity than that used in the other two test series and the reaction will evidently terminate whenever the ferro-complex (which in this case acts as the oxidizing agent) has been used up.

The maximal luminescence intensity values (G_m) and light sum values (L) obtained with these reaction compounds without inhibiting agents have been noted (in relative units) in Table I. Largest maximal luminescence intensity was obtained with hydrogen peroxide present and at highest alkaline concentration, whereas the largest light sum value is shown for an absence of H_2O_2 , i.e. for largest Luminol quantities. This shows that the greater reaction speed of the catalytic reaction produces a more intensive luminescence with shorter duration of reaction, while the non-catalytic reaction shows lower light intensity but yields a larger quantity of luminescence radiation, due to longer duration of this reaction.

Hydrochinon shows a distinctly inhibiting effect on the non-catalytic reaction: even in very low concentration this inhibiting agent will reduce the maximal intensity of luminescence, as well as the light sum. Figure 1 shows a number of decay curves of luminescence (intensity-time curves) for various concentrations of hydrochinon. It may be seen that in the case of higher hydrochinon concentration maximal intensity of luminescence will be reached only after a certain reaction time has passed.

Table I

Intensity of luminescence and light sum values for reaction compounds containing no inhibiting agents

Reaction Compounds	G_m	L
without H_2O_2 (non-catalytic)	57	141.5
(catalytic) with H_2O_2 NaOH: 0.005 mol/l	11.4	5.4
(catalytic) with H_2O_2 NaOH: 0.045 mol/l	122	24.8

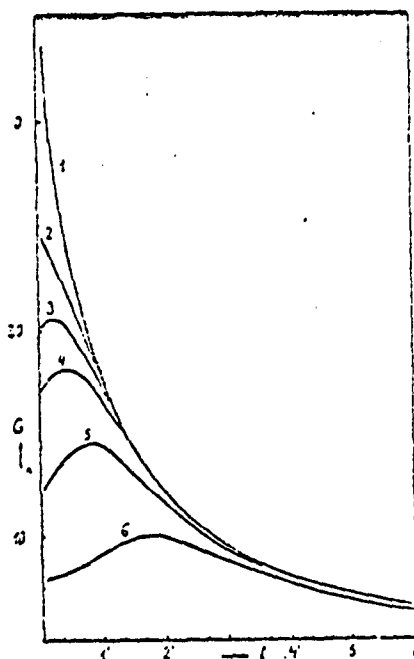


Figure 1. Inhibition of the non-catalytic reaction by hydroquinone. Inhibitor concentration $\times 10^8$ mol/l: 1. -- 2. 5.4 3. 9 4. 18 5. 36 6. 90

The initial and the maximal intensity may be computed for each inhibitor concentration from these decay curves, also

the light sum of luminescence for the reaction compounds without inhibitor addition. The numerical values compiled in Tables II, III and IV were obtained in this manner. These values may be compared with one another, independent of the absolute values of luminescence intensities. They refer to the non-catalytic, as well as the catalytic type of reaction obtained from the compounds with component listings given in 1., 2. and 3. These tables also show the values for the general inhibitor constant β (see publication VII). It may be seen that for compounds 1. and 2. the reciprocal value of the constant ($1/\beta$) corresponds to the half-value concentration of inhibition (\bar{c}), which was computed by means of graphical interpolation. This means that this type of reaction well satisfies the inhibitor equation. No constancy of the β -values was obtained, however, for compound 3: inhibition increases at a much higher rate than would correspond to the inhibitor equation. The high alkaline concentration in this case seems to entail secondary influences (rapid oxidizing of the inhibitor).

Table II

Luminol + $K_3\sqrt{Fe(CN)_6}$

Compound 1

Hydrochinon Mol/l. $\cdot 10^3$	G in %		L in %	$\beta \cdot 10^{-6}$
	Anfangs- ^②	Maximal-		
	b) Intensität			
—	100	100	100	—
5,4	68,1	63,1	93,0	8,7
9,0	53,5	54,4	89,4	9,7
18	33,4	45,6	86,6	11,1
36	25,4	23,0	81,0	8,2
90	10,4	17,5	56,3	9,6

c) Mittelwert: $\beta = 9,5 \cdot 10^{-6}$; $1/\beta = 1,06 \cdot 10^{-7}$; $\bar{c} = 1,10 \cdot 10^{-7}$

Legend: a) Initial; b) Intensity c)
Mean values.

Table III

Luminol + H_2O_2 + $K_3\sqrt{Fe(CN)_6}$ ₇
 NaOH: 5×10^{-3} mol/l

Compound 2

Hydrochinon Mol/l. 10^4	G in %		L in %	$\beta \cdot 10^{-4}$
	Anfangs- ^{a)}	Maximal-		
	b) Intensität			
—	100	100	100	—
0,9	85,9	85,9	335,7	1,8
1,6	64,9	64,9	459,8	3,0
2,7	59,7	59,7	592,5	2,5
3,6	22,8	24,9	481,0	9,4
4,5	3,8	12,1	117,1	5,6

c) Mittelwert: $\beta = 4,1 \cdot 10^{-4}$; $\beta_1 = 2,45 \cdot 10^{-4}$; $\bar{c} = 2,4 \cdot 10^{-4}$

Legend same as in Table II

Table IV

Luminol + H_2O_2 + $K_3\sqrt{Fe(CN)_6}$ ₇
 NaOH: 4.5×10^{-2} mol/l

Compound 3

Hydrochinon. Mol/l. 10^4	G in %		L in %	$\beta \cdot 10^{-4}$
	Anfangs- ^{a)}	Maximal-		
	b) Intensität			
—	100	100	100	—
0,9	99,2	99,2	267,3	0,9
1,6	80,3	80,3	293,7	1,3
2,16	49,4	49,4	189,0	5,0
2,52	30,1	30,4	149,8	9,2
2,56	14,1	14,1	62,3	20,0
2,61	2,4	2,4	34,2	140

$\bar{c} = 2,24 \cdot 10^{-4}$

Legend same as in Table II

The essential fact is presented by the extremely low half-value concentration of hydrochinon in the non-catalytic reaction. In order of magnitude it is approximately in a relationship of 1: 4,000, compared to the 1: 20,000 concentration of $K_3\sqrt{Fe(CN)_6}$ ₇, or of the Luminol, respectively,

contained in this compound. This means, however, that with this inhibitor concentration one half of the excited (stimulated) Luminol molecules (ions), capable of emitting one quantum of light, will be prevented from emitting if only one molecule (ion) of hydrochinon is present in a solution containing 4,000 molecules of the ferro-complex and 20,000 molecules of Luminol. This represents a very effective inhibition which is only rarely observed in the other reactions and indicates a particular reaction mechanism for the non-catalytic type of reaction. In the usual thermal and photochemical reactions, as well as in the extinguishing of fluorescence caused by foreign additions the half-value concentration of the inhibiting agent is frequently higher (by a ratio of 1 : 100) than the concentration of the reaction components, while reactions for which a chain mechanism is assumed are generally more effectively inhibited [See Note 7].

[Note: 7] We refer to the inhibiting of autoxidizing of benzaldehyde by hydrochinon and similar substances [4], inhibition of autoxydizing of cystein by $MnSO_4$ [5], retardation of photopolymerizing of styrol and Vinylacetate by hydrochinon [6] and cancellation of chemoluminescence os phosphorus by turpentine oil vapors (GRAHAM 1829).)

The other inhibitors tested by us also show less effectiveness on the non-catalytic reaction. Generally, speaking, however, it could be noted that this type of reaction is more easily inhibited than the catalytic type (see Table V). Potassium iodide, on the other hand, causes an increase in the maximal intensity of luminescence (probably due to a primary electrolytic effect) which also results in a slight increase in the light sum. The molar concentration of KI causing an increase of maximal intensity of luminescence to twice its original value are also given in Table V.

For an explanation of the very effective inhibition described above we may assume that in the chemical reaction of the potassium-ferro(III)cyanide with the Luminol not really the hexacyanide ion of the ferro-complex is acting but rather the pentacyanide ion. This latter ion is formed by hydrolysis (in small concentration) from the hexacyanide, according to the following reaction equation:

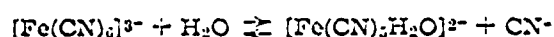


Table V

Half-value concentrations of various inhibitors

Inhibitor	a) Reaktion ohne H_2O_2	b) mit H_2O_2	
		0,005 M NaOH	0,045 M NaOH
	c		
Phenol	$2,62 \cdot 10^{-3}$	$3,30 \cdot 10^{-3}$	$2,70 \cdot 10^{-3}$
Hydrochinon	$1,16 \cdot 10^{-3}$	$2,40 \cdot 10^{-3}$	$2,24 \cdot 10^{-3}$
Nicot	$7,70 \cdot 10^{-4}$	$6,07 \cdot 10^{-4}$	$3,42 \cdot 10^{-3}$
Anilin	$1,03 \cdot 10^{-3}$	$2,60 \cdot 10^{-3}$	$5,30 \cdot 10^{-3}$
	d) Konzentration in Mol/l die G_m um 100% erhöht		
Elektrolyt			
c) KJ	0,117	0,018	0,032

Legend: a) Reaction w/o H_2O_2 ; b) With H_2O_2 ;
c) KI; d) Concentration in mol/l causing 100%
increase in G_m .

Presence of the aquo-pentacyanide ion in watery solutions has been observed repeatedly under varying test conditions and it seems probable that the labile binding of the water molecule may be easily replaced in this ion by a Luminol ion or molecule. This would mean that the Luminol-complex of the pentacyanide constitutes the critical reaction complex (unstable interim product) for the non-catalytic Luminol reaction. Inhibition would then be caused by displacement of the Luminol ion in this critical complex by a hydroquinon ion which will prevent the reaction leading to light emission. It appears that other thermal reactions are taking place simultaneously which also do not cause light emission and thus result in an appreciable decrease in the luminescence light sum value.

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